HEK293 HCP ELISA Kit

Catalog Number: HH20111B-96

【INTENDED USE】

The HEK293 HCP (Human Embryonic Kidney 293 cell host proteins) residue detection kit (enzyme-linked immunosorbent assay) produced by our company is a high-sensitivity universal HEK293 HCP residue detection kit, which can sensitively, specifically and accurately detect the residue of HEK293 cell host proteins in different process stages of biological products.

【 PRINCIPLE】

This kit uses a double antibody sandwich enzyme-linked immunosorbent assay to determine the residual amount of HEK293 HCP in samples. The goat polyclonal antibody against HEK293 HCP is pre-coated on the microtiter plate. Add standards and samples to the wells, and then add horseradish peroxidase-labeled polyclonal antibody against HEK293 HCP to the wells. At this time, the HEK293 HCP in the sample is captured by the immobilized capture antibody and binds to the enzyme-labeled antibody to form an antibody-antigen-antibody "sandwich complex". Subsequently, color development is performed by adding TMB substrate, and the absorbance (OD value) is measured at wavelengths of 450 nm/630 nm using an enzyme labeler. The absorbance is positively correlated with the content of host cell proteins in the sample.

【 PRODUCT PERFORMANCE INDICATORS】

Quantitative limit: 5 ng/mL

Linear range: 5-810 ng/mL

Accuracy (reciprocal recovery rate): 80%-120%

Accuracy (measurement deviation): ≤| ±15% |

Repeatability (within batch): ≤10%

【MATERIALS PROVIDED】

Number	Component	Size	Storage Condition
1	Microtiter Plate	96T	2-8℃
2	Standard（81000 ng/mL）	200 μL×1 tube	2-8℃
3	Sample Dilution	30 mL×1 vial	2-8℃
4	HRP-Conjugate Antibody（100×）	200 μL×1 tube	2-8℃(keep in dark)
5	HRP-Conjugate Antibody Dilution	15 mL×1 vial	2-8℃
6	Wash Buffer (20×)	30 mL× 1 vial	2-8℃
7	Developing solution A	8 mL× 1 vial	2-8℃ (keep in dark)
8	Developing solution B	8 mL× 1 vial	2-8℃ (keep in dark)

9	Stop Solution	15 mL× 1 vial	2-8℃
10	Microplate Sealers	3	-

【 STORAGE】

Unopened Kit is stored at 2-8℃ and the kit is stable for 12 months. The production date and expiration date are shown on the kit label.

【REAGENTS/EQUIPMENTS REQUIRED BUT NOT SUPPLIED】

1. Enzyme-labeled instrument, thermostatic oscillator (or thermostatic incubator), plate washer (or hand washing), vortex oscillator, timer.
2. Pipettes and pipette tips (0.5- 10 μL, 10- 100 μL, 30-300 μL, 100- 1000 μL).
3. Deionized or distilled water, bibulous paper, EP tubes.

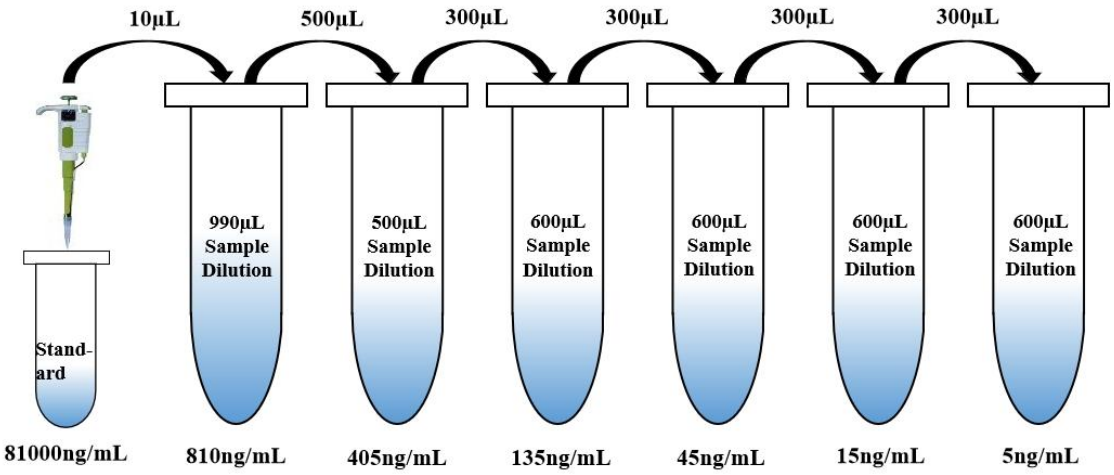
【REAGENT PREPARATION】

- Wash Buffer (1×): add the deionized water into the Wash Buffer (20×) to dilute for 20 times for standby, for example, add 190 mL of deionized water into 10 mL Wash Buffer (20×), and mix well.If there is crystallization in the wash buffer (20×), it should be gently shaken at room temperature or 37℃ in a water bath, and then diluted after the crystallization is completely dissolved. Unused wash buffer (20×) should be stored at 2-8℃.
- HRP-Conjugate Antibody (1×): Dilute the HRP-Conjugate Antibody (100×) with HRP-Conjugate Antibody Dilution 100 times for standby.
- Substrate Solution: Mix the developing solution A and developing solution B in equal volume before 10 minutes using. Avoid light to ensure that the substrate solution is not contaminated. If the substrate solution after mixing has turned blue, do not use it.

【ASSAY PROCEDURE】

Bring all reagents and samples to room temperature before use.

1. Bring the test slats from the aluminum foil bag that has been balanced to room temperature, put the remaining slats back into the aluminum foil bag, seal them, and store them in 2-8℃.
2. The standard (81000 ng/mL) was diluted with the sample dilution to 810ng/mL, then diluted twice to 405ng/mL, and then prepared as a standard by triple gradient dilution, as shown in the figure below:



3. Sample incubation: add the prepared standards and the samples to be tested into the ELISA plate (the recommended adding sequence: standard well, blank well, sample well, spiked sample well, and the standards shall be added as per the concentration gradient), 50 μL/well.

4. HRP-conjugate antibody incubate: Add 100μL of HRP-conjugate antibody each well, and then shake and incubate at 500 rpm, 37℃ for 2h.

5. Washing board: discard the liquid in the well and wash the board with Wash Buffer (1×) for 3 times (250 μL /well). Pat dry residual liquid in sample well. (Before discarding the liquid in the well each time, if washing plate by hand, the liquid can be left for 1min after adding and slightly shaken; If plate washing machine is used, 5s can be slightly shaken after adding wash buffer).

6. Color development: the pre-prepared substrate solution is added to the enzyme label plate, mixed well (100 μL/well), and incubated in darkness for 30min.

7. Termination: add stop solution (100 μL /well).

8. Recording: The OD value at 450 nm/630 nm should be measured by placing the plate in the microplate reader, and the reading should be completed immediately.

【CALCULATION OF RESULTS】

1. Calculation of absorbance value: The calibrated value of light absorbance of each standard or sample is: ($OD_{450\ nm}$-$OD_{630\ nm}$)- absorbance value of the blank control well ($OD_{450\ nm}$-$OD_{630\ nm}$) .

2. Plot the standard curve with concentration of the standard as the horizontal coordinate (X) and the calibrated value of light absorption of the standard as the vertical coordinate (Y), and a four-parameter Logistic mathematical model is recommended to fit the equation: Y=（（A-D）/（1+（X/C）^B））+D. Substitute OD value of the sample ($OD_{450\ nm}$ - $OD_{630\ nm}$)- absorbance value of the blank control well ($OD_{450\ nm}$-$OD_{630\ nm}$))

3. If the OD value of the sample to be tested exceeds the highest OD value of the standard curve, the sample shall be redetermined after dilution.

【 PRECAUTIONS】

1. All the components in the kit must be restored to room temperature (20-25℃) before use.
2. Each component shall be mixed well before use, to guarantee uniformity of the reagent, the standards shall be centrifuged for 5 s temporarily, and centralized the liquid on the tube wall and cover onto the bottom of the tube, and place all the reagents back to 2-8℃ immediately after use.
3. The kit must be used within the shelf life, and corresponding standard curve shall be prepared for each test. Mixed use of different batches of related reagents is not recommended.
4. Be careful not to touch the bottom of the microplate during adding liquid to the ELISA plate, to prevent damaging the coating layer. Replace the reagent reservoirs and the tips timely between different samples and steps, to avoid cross contamination.
5. During beating the strips after washing, take care to prevent the strips from shedding, and the plate sealers shall not be used repeatedly.
6. Black flocs may be generated at high concentration during color development, this is a normal phenomenon, it is minor in degree, it may not affect the final reading results.
7. Note to check whether the correct detection wavelength and fitting equation are selected during reading.
8. The best detection results can only be guaranteed by strictly following the instructions and using all the reagents supplied with the kit.
9. The difference in testing results can be caused by several factors, including operations of the experimenters, usage way of the pipettes, plate washing method, reaction time or temperature, storage of the kit etc.
10. Our company is only responsible for the kit itself, but not be responsible for sample consumption caused by using this kit. Please fully consider the possible usage amount of samples before use, and reserve sufficient samples.
11. This kit is only used for in study, not for clinical diagnosis.

【SAFETY INSTRUCTIONS】

1. All the biological samples have potential biosafety risks, and the users shall operate, dispose of and discard the samples in strict accordance with the local laws and relevant provisions.

2. For safety reasons, the operators shall wear the personal protective equipment, such as lab coats, gloves, face masks and safety glasses.

【 PROBLEM ANALYSIS】

If there is any problem with the experimental results, please take photos of the color rendering results in time, and properly preserve the unused slats and reagents. Then contact technical support to find out the reason.

Problem Description	Possible Reasons	Solution
Gradient difference of standard curve	Dilution error	The standard curve is not diluted according to the dilution factor
	The liquids are aspirated or added inaccurately	Check the pipette and tips
	The ELISA plates are not washed completely	Guarantee the plate washing times, and the amount used for washing liquid per well
The color development is weak or colorless	The incubation time is too short	Guarantee sufficient incubation time
	The experiment temperature is not correct	Use the recommended incubation temperature
	The reagent volume is insufficient or miss to add the reagent	Check the liquid aspiration and adding process, and guarantee all the reagents are added sufficiently in sequence
	The developing solution are prepared wrongly	The developing solutions A and B are mixed in same volume and protected from light, which are prepared 10 min before color development
The OD value are of low reading	The sample is not treated	The samples are tested after centrifugation by heating
	The plate reader are set incorrectly	Check the wavelength and optical filter device on the plate reader
		The plate reader shall be opened in advance before reading for preheating
The OD value are of high reading	The sample is not diluted	It is recommended to validate the dilution factor of the sample
The coefficient of variation (CV value) is large	The liquid adding is incorrect	Check the liquid adding condition
	There are pollution at the bottom of the ELISA plate	Check the bottom of the ELISA plate for residual liquid or handprint
	There are foreign matters or bubbles in the plate well	Confirm that there are no foreign matters in the plate well before adding samples, and confirm that there are no bubbles after adding samples
	The plate is not sealed or sealed incompletely during incubation	Seal the plate with a plate sealer
The background value is high	The ELISA plates are not washed completely	Wash the plate according to the method recommended in the instruction for use
		If automatic plate washer is used, please check all the liquid adding port and waste liquid outlet for blocking
		If the plate is washed by hands, the plate washing times can be added properly
		Insufficient washing or missing to wash may lead to high background
	Incubation time and temperature	Operate in strict accordance with requirements in the instruction for use
	Consumption pollution	The consumables used (such as tubes, tips etc.) are not clean
	The washing solution is polluted	Prepare fresh washing solution

	The developing solution is polluted	The color developing solution itself does not have colors, please ensure that the color developing solution is not polluted by metal ion or oxidizing agent before use, and it is protected from light.
The sensitivity is low	The kit is stored improperly	Store the related reagents in accordance with the requirements in the instruction for use

The specification is issued in version A/0 with an effective date of 2025/10/10.

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